Br. J. Pharmacol. (1992), **107**, 970–975

Ramipril prevents left ventricular hypertrophy with myocardial fibrosis without blood pressure reduction: a one year study in rats

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1 Angiotensin converting enzyme (ACE)-inhibitors have been demonstrated to be effective in the treatment of cardiac hypertrophy when used in antihypertensive doses. The aim of our one year study with an ACE-inhibitor in rats was to separate local cardiac effects produced by a non-antihypertensive dose from those on systemic blood pressure when an antihypertensive dose was used.

2 Rats made hypertensive by aortic banding were subjected to chronic oral treatment for one year with an antihypertensive dose of the ACE inhibitor, ramipril 1 mg kg^{-1} daily, (RA 1 mg) or received a low dose of 10 $\mu g\ kg^{-1}$ daily (RA 10 μg) which did not affect high blood pressure.

3 Chronic treatment with the ACE-inhibitor prevented left ventricular hypertrophy in the antihypertensive rats as did the low dose which had no effects on blood pressure. Similar effects were observed on myocardial fibrosis. Plasma ACE activity was inhibited in the RA 1 mg but not in the RA 10 μg group although conversion of angiotensin (Ang) I to Ang II in isolated aortic strips was suppressed in both treated groups. Plasma catecholamines were increased in the untreated control group, but treatment with either dose of ramipril normalized the values. The myocardial phosphocreatine to ATP ratio (an indicator of the energy state in the heart) was reduced in the vehicle control group whereas the hearts from treated animals showed a normal ratio comparable to hearts from sham-operated animals.

4 After one year, five animals were separated from each group, treatment withdrawn, and housed for additional six months. In the RA 1 mg group, blood pressure did not reach the value of the control vehicle group and surprisingly, left ventricular hypertrophy and myocardial fibrosis did not recur in animals during withdrawal of treatment.

5 These data show that long term ACE inhibitor treatment with ramipril in antihypertensive and non-antihypertensive doses prevented cardiac hypertrophy and myocardial fibrosis. This protective effect was still present after 6 months treatment withdrawal.

Keywords: Aortic banding; cardiac hypertrophy; myocardial fibrosis; long term treatment; angiotensin converting enzyme inhibition; low dose ramipril

Introduction

Left ventricular hypertrophy (LVH) is regarded as an independent risk factor in hypertensive patients. It is associated with increased incidence of cardiac failure, myocardial infarction, severe arrhythmias and sudden death (Messerli & Ketelhut, 1991). Clinical data indicate, that reversal of LVH may improve the prognosis of hypertensive patients (Dahlöf *et al.*, 1992). It is also known, that therapeutic approaches differ in the regression of this disorder despite application of equihypotensive doses of such antihypertensives (Hill *et al.*, 1979).

The renin angiotensin system (RAS) is involved in the development and maintenance of hypertension and cardiac hypertrophy (Hall & Karlberg, 1986), and angiotensin converting enzyme (ACE) gene expression in the heart is induced in LVH (Schunkert *et al.*, 1990). Furthermore in hypertrophied myocardium following left ventricular infarction, increased ACE activity was measured (Johnston *et al.*, 1991). A direct role of angiotensin II (Ang II) as a myocardial growth factor seems probable (Schelling *et al.*, 1991).

In experimental and clinical studies ACE inhibitors have been demonstrated to be effective in the treatment of cardiac hypertrophy when used in antihypertensive doses. The aim of our one year study in rats made hypertensive by aortic banding was to separate cardiac effects of the ACE inhibitor, ramipril, from those on systemic blood pressure by use of doses with and without effect on hypertension.

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Methods

Adult male Sprague Dawley rats weighing 270–280 g (Möllegaard, Skensved, Denmark) were fasted for 12 h before surgery. Anaesthesia was induced by i.p. injection of 200 mg kg^{-1} hexobarbitone (Evipan). The abdomen was opened by a parallel cut to the linea alba. The abdominal aorta was exposed above the left renal artery and a silk thread was passed under it. A cannula no. 1 (0.9 × 40 mm) was placed longitudinally to the aorta and both aorta and cannula were tied. The cannula was then removed, leaving an aortic lumen determined by the diameter of the cannula. Before the abdomen was closed with catgut, the animals received 5.5 mg rolitetracycline (Reverin, Hoechst AG, Frankfurt, Germany). The skin was closed by clipping and covered with tar spray. Sham-operated animals were subjected to the same procedure, but without aortic banding.

During the first 5 days following the operation the animals received rolitetracycline (1 g 350 ml^{-1}) in the drinking water.

The animals were alloted to four groups of 30 rats each as follows: Group I, sham-operated; Group II, aortic banding without treatment; Group III, aortic banding, and ramipril treatment with 1 mg kg^{-1} day^{-1}, and antihypertensive dose; Group IV, aortic banding, and ramipril treatment with 10 μg kg^{-1} day^{-1}, a non-antihypertensive dose. Ramipril treatment

started the day after the operation and continued for one year by daily oral gavage. The animals were weighed weekly.

Final examinations

At the end of the experiments after one year the animals were anaesthetized with hexobarbitone (200 mg kg^{-1} i.p.) and blood pressure measured via catheters in the left carotid artery. Blood pressure measurements in concious rats by conventional tail-cuff methods were not possible, since there was a large drop of blood pressure distal to the ligature; hence, we had only one measurement at the end of the study. However, we have observed in normal rats that blood pressure values measured under hexobarbitone anaesthesia are not different from those measured in concious rats with a photoelectric tail-cuff pulse detector. This was also reported by other groups when sodium-pentobarbitone anaesthesia was used (Owen & Reidy, 1985).

The hearts were excised, cleaned of blood with saline, gently blotted to dryness, and the total cardiac mass, left ventricular weight (LVW) including the septum as well as the remaining cardiac tissue representing the right ventricle (RVW) were determined (to the nearest 0.1 mg). Weights are given per 100 g body weight.

Biochemistry

In the thoracic aorta, basal guanosine 3':5'-cyclic monophosphate (cyclic GMP) content was determined by radioimmunoassay (New England Nuclear, Dreieich, Germany). Cyclic GMP content was expressed as pmol mg^{-1} protein. Hearts for measurements of ATP, phosphocreatine and glycogen (5 to 8 hearts per group) were quickly removed and placed in liquid nitrogen. Thereafter in the left ventricular tissue, phosphocreatine (PCr), ATP and glycogen were determined (Linz *et al.*, 1989). From PCr and ATP values the PCr to ATP ratio was calculated.

Plasma renin-activity was measured by incubation of 25 µl of rat plasma with an excess of renin substrate. Plasma ACE-activity was determined radioenzymatically with [^{3}H]-Hip-Gly-Gly as substrate (Waeber *et al.*, 1989). Plasma catecholamine content of noradrenaline and adrenaline was measured by high performance liquid chromatography (h.p.l.c.).

Morphological studies

Left ventricular tissue was stained for fibronectin by a specific monoclonal antibody (ICN Biologicals, Lisle, United Kingdom).

Frozen sections of 4 µm thickness were fixed for 10 min at −20°C in cold acetone, rinsed and incubated with the first antibody during 1 h in a moist chamber. After rinsing with buffer, the biotinylated antirabbit IgG (Amersham, United Kingdom) was added to the sections. Fluorisothiocyanat labelled spectravidin was finally added after rinsing and the sections were mounted with Mowiol. The sections were viewed in an Olympus Vanox T microscope equipped for fluorescence microscopy and connected to an automatic videorecording system (VIPER, Gesotec, Darmstadt, Germany). The pictures were digitized and the degree of fibrosis automatically determined as a percentage of the entire tissue section. According to morphometric principles, these percentage values are representative for the entire left ventricle (Weibel, 1969).

Functional studies

In the isolated aorta the contractile response to Ang I or Ang II and the relaxing effect of bradykinin (BK-endothelium dependent relaxation) was tested. Intact proximal parts of the thoracic aorta were sectioned into 2 mm wide rings, cut off, and suspended at 1 g tension in 25 ml organ chambers filled with a buffer solution at 37°C of the following composition (mM): NaCl 113.8, $NaHCO_3$ 22, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.1, $CaCl_2$ 2.5 and glucose 5.5, gassed with a 95% O_2:5% CO_2 mixture to give pH 7.4. After 1 h, when a stable contractile tone had been established, Ang I (1×10^{-8} to 1×10^{-6} mol l^{-1}) was added to test the ability of the tissue ACE to convert Ang I into Ang II. Contraction of aortic strips was registered in mN. After a wash-out period of 10 min, noradrenaline was added for a final concentration of 1×10^{-8} mol l^{-1}, which produced a stable submaximum isotonic contraction. Then BK was added to give final concentrations of 1×10^{-8} to 1×10^{-6} mol l^{-1}. Relaxation of aortic strips was assessed as percentage decrease in contraction. The maximal response to Ang I was observed in aortic strips from sham-operated animals at 1×10^{-7} mol l^{-1} and the maximal relaxation we found in these aortic strips at 1×10^{-7} mol l^{-1} BK.

Withdrawal

After one year five animals were separated from each group and treatment was stopped. After an additional six months blood pressure, heart weight, left ventricular weight and myocardial fibrosis were determined; in addition functional and biochemical studies were carried out (see above).

Ramipril was synthetized in the Pharma Synthesis, Hoechst AG, and dissolved in saline. Angiotensin I and bradykinin were purchased from Sigma Chemicals, München, Germany.

Statistical analysis

Statistical analysis of the data was performed with ANOVA and Bonferroni Test when appropriate. Differences were considered significant if $P < 0.05$. Results are given as mean ± s.d.

Results

At the end of the year, plasma ACE activity and renin activity were measured. After one year of aortic banding and ramipril treatment, plasma renin activities were not changed in the animals (Table 1). Plasma ACE activity was not different in sham-operated animals and in control vehicle animals. However, plasma ACE activity was inhibited in the group which received the 1 mg kg^{-1} dose of ramipril whereas plasma ACE activity was not reduced by the lower dose of 10 µg kg^{-1} (Table 1).

To estimate tissue ACE activity, isolated aortae from all groups were exposed to 1×10^{-7} mol l^{-1} Ang I to measure the Ang II-induced contraction of the blood vessels after conversion of Ang I to Ang II by tissue ACE. In both ramipril groups the contraction of the aortae after Ang I application was significantly reduced in comparison to the sham and control vehicle group (Figure 1).

After contraction with noradrenaline, BK induced a relaxation of 45 ± 5% on aortic strips from sham-operated animals. Subjecting rats to aortic banding reduced the endothelium-dependent relaxation of these blood vessels to 12 ± 3% when exposed to BK. However, this effect was prevented by treating the animals with either 1 mg kg^{-1} or 10 µg kg^{-1} ramipril, which preserved the BK-induced relaxation at 40 ± 4% and 38 ± 5% respectively.

In sham-operated animals, aortic cyclic GMP tissue content was 58.2 ± 6.9 fmol mg^{-1} protein. Cyclic GMP was significantly lowered in the control vehicle group (37.6 ± 2.7 fmol mg^{-1} protein); however, ramipril treatment in both doses increased cyclic GMP to values above those in sham-operated animals (RA 1 mg: 95.6 ± 17.6 fmol mg^{-1} protein; RA 10 µg 72.4 ± 15.3 fmol mg^{-1} protein).

Mean arterial blood pressure (MAP) was increased after aortic banding. This effect was completely abolished by the higher dose of 1 mg kg^{-1} ramipril known to have blood

Table 1 Oral treatment for one year with ramipril 1 mg or 10 μg kg^{-1} day^{-1} in rats with aortic banding

	Sham	*Vehic con*	*RA 1 mg*	*RA 10 μg*
PRA (ng AI ml^{-1} h^{-1})	7.3 ± 1.4	5.3 ± 1.2	7.2 ± 1.5	5.4 ± 1.1
PACEA (nmol min^{-1} ml^{-1})	148 ± 9	166 ± 9	17 ± 7*	132 ± 8
HW (mg 100 g^{-1} b.wt.)	300 ± 3	393 ± 5*	294 ± 5	309 ± 6
PAd (nmol l^{-1})	9.1 ± 1.7	19.9 ± 2.8*	8.9 ± 1.5	10.2 ± 2.1
ATP (μmol g^{-1} wet wt.)	5.8 ± 0.5	5.0 ± 0.3*	5.7 ± 0.4	5.6 ± 0.4
PCr (μmol g^{-1} wet wt.)	7.9 ± 0.5	4.2 ± 0.4*	7.6 ± 0.4	7.7 ± 0.3
Glycogen (μmol g^{-1} wet wt.)	26.6 ± 3.3	27.5 ± 3.5	25.8 ± 3.7	25.9 ± 4

Plasma renin activity (PRA), plasma ACE activity (PACEA), heart weight (HW), plasma adrenaline (PAd), heart-ATP, -phosphocreatine (PCr), and -glycogen content. Vehic con: vehicle control group.
*$P<0.05$ versus sham-operated group.

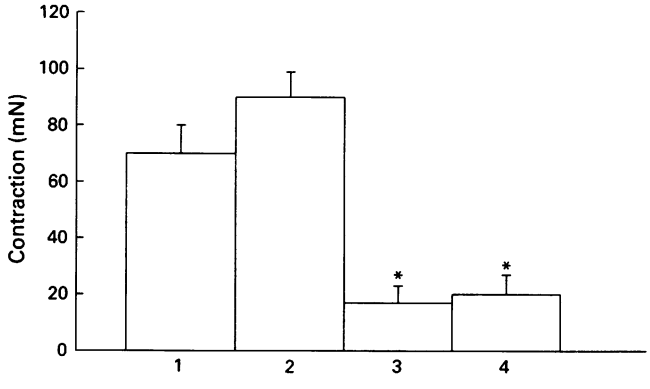

Figure 1 Tissue angiotensin-converting enzyme activity reflected as conversion of angiotensin (Ang) I (1×10^{-7} mol l^{-1}) to Ang II in isolated aortic strips (contraction in mN) from rats treated for one year with ramipril 1 mg (3) or 10 μg kg^{-1} day^{-1} (4). *n*: 15–22 per group. *$P<0.05$ vs sham (1). Vehicle control (2).

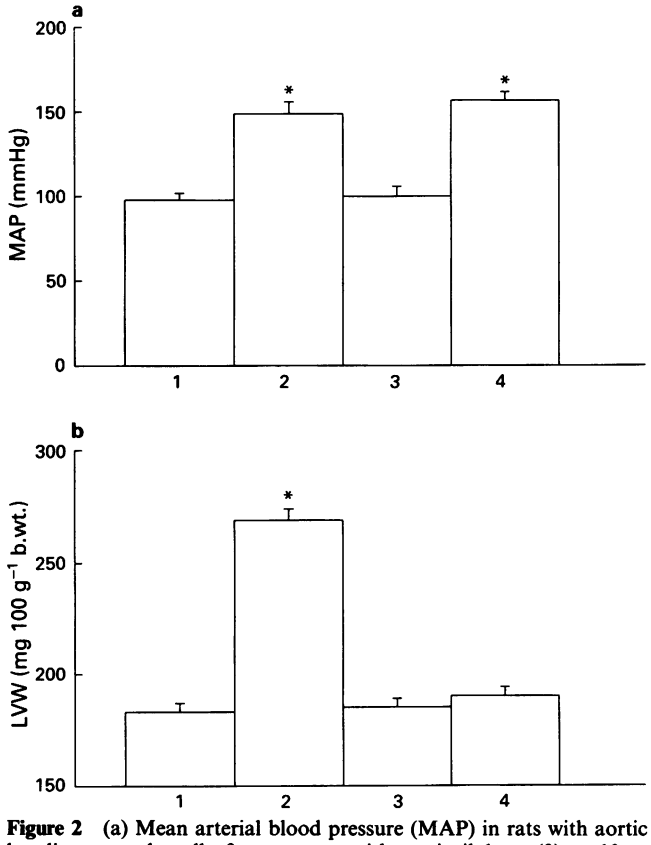

Figure 2 (a) Mean arterial blood pressure (MAP) in rats with aortic banding treated orally for one year with ramipril 1 mg (3) or 10 μg kg^{-1} day^{-1} (4). *n*: 15–22 per group. (b) Left ventricular weight (LVW) in rats with aortic banding treated orally for one year with ramipril 1 mg (3) or 10 μg kg^{-1} day^{-1} (4). *n*: 15–22 per group. *$P<0.05$ vs sham (1). vehicle control (2).

pressure lowering effects, whereas the lower dose of 10 μg kg^{-1} ramipril did not decrease blood pressure (Figure 2a).

A clear dissociation between blood pressure and left ventricular weight was found in the low dose RA group which showed no ventricular hypertrophy although hypertension was not prevented. Left ventricular weight in the high dose RA group did not differ from sham-operated matching controls, whereas hearts from control vehicle-treated animals showed ventricular hypertrophy (Figure 2b). Right ventricular weight (44–54 mg 100 g^{-1} b.wt.) and body weight (490–520 g) were not different in all four groups.

In line with the values for left ventricular hypertrophy are the observations on the occurrence of myocardial fibrosis: this was not seen in hearts from animals treated with the higher as well as the lower dose of ramipril, whereas in hearts from rats with aortic banding and treated with vehicle, myocardial fibrosis occurred (Figure 3).

Plasma noradrenaline increased from 2.3 ± 0.9 nmol l^{-1} in sham-operated animals to 5.4 ± 1.2 nmol l^{-1} following aortic banding, whereas the values were not significantly different from the sham group (2.8 ± 1.0 and 2.2 ± 0.8 nmol l^{-1}, respectively) for 1 mg kg^{-1} and 10 μg kg^{-1} ramipril. Plasma adrenaline values were comparable (Table 1).

The phosphocreatine to ATP ratio (Table 1) was reduced in hypertrophied hearts from rats of the vehicle control group (0.84), whereas ACE inhibitor treatment improved the ratio (RA 1 mg: 1.34 and RA 10 μg: 1.37) compared to the ratio found in hearts from sham-operated animals (1.34).

Withdrawal of the treatment did not change left ventricular weight to body ratio in the different groups (Figure 4b), and in the earlier RA 1 mg group (with prevention of hypertension) blood pressure did not reach the value of the stenosis vehicle group (Figure 4a).

Myocardial fibrosis did not occur after six months' withdrawal of ACE inhibitor treatment (Table 2).

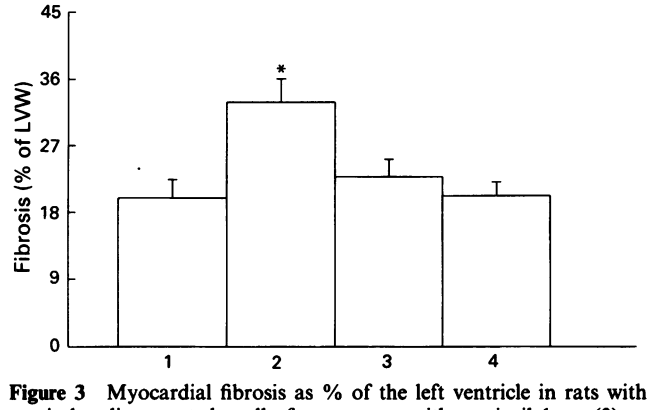

Figure 3 Myocardial fibrosis as % of the left ventricle in rats with aortic banding treated orally for one year with ramipril 1 mg (3) or 10 μg kg^{-1} day^{-1} (4). *n*: 15–22 per group; *$P<0.05$ vs sham (1). vehicle control (2).

Table 2 Withdrawal of ramipril treatment after one year for six months

	Sham	*Vehic con*	*RA 1 mg*	*RA 10 μg*
Fib % LVW	22.9 ± 1.5	36 ± 2*	23.5 ± 1.8	24.9 ± 2
Ang I to Ang II (contraction in mN)	75 ± 5	86 ± 8	61 ± 6	66 ± 5
BK (relaxation in %)	48 ± 4	9 ± 3*	46 ± 5	45 ± 4
cGMP (fmol mg^{-1} prot)	64 ± 6	36 ± 4*	83 ± 7	78 ± 6

Fibrosis % of left ventricular weight (Fib % LVW), conversion of angiotensin (Ang) I to Ang II in isolated aortic rings, relaxing effects by bradykinin (BK), basal cyclic GMP (cGMP) content in rings of rat aorta. Vehic con: vehicle control group. *$P<0.05$ versus sham operated group.

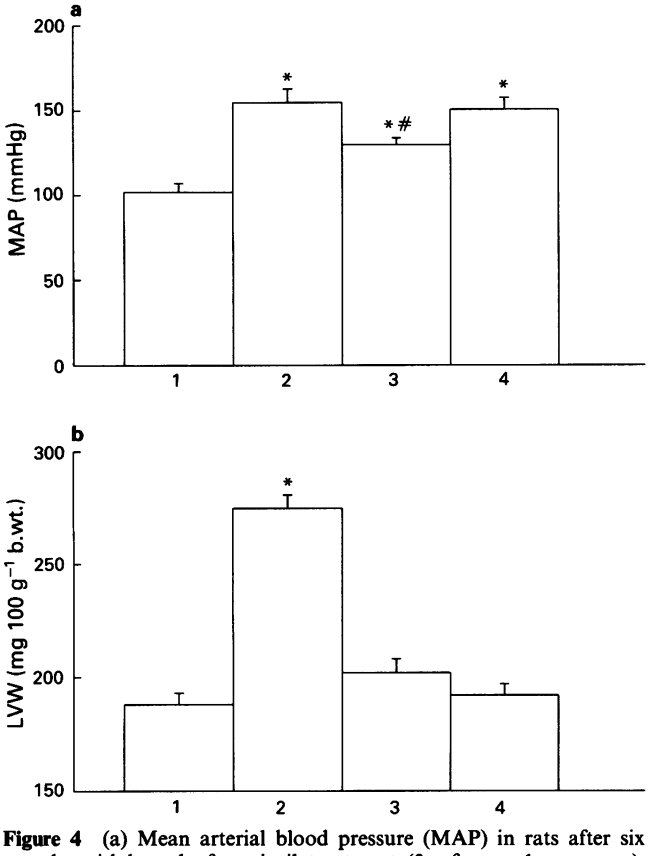

Figure 4 (a) Mean arterial blood pressure (MAP) in rats after six months withdrawal of ramipril treatment (3 = former 1 mg group), (4 = former 10 μg group). *n*: 5 per group. *$P<0.05$ vs sham (1); #$P<0.05$ vs vehicle control (2). (b) Left ventricular weight (LVW) in rats after six months withdrawal of ramipril treatment (3 = former 1 mg group), (4 = former 10 μg group) *n*: 5 per group. *$P<0.05$ vs sham (1). Vehicle control (2).

The conversion of Ang I to Ang II was not different between the groups, but the impaired relaxing effect in the former untreated control group was still present whereas aortic strips of both the earlier treated groups relaxed normally (Table 2). These relaxations were accompanied by elevated basal cyclic GMP levels in strips from previously treated animals (Table 2).

Discussion

The present study shows that long term ACE inhibitor treatment with ramipril over one year in antihypertensive and non-antihypertensive doses prevents LVH and myocardial fibrosis. Earlier experimental studies had shown that ACE inhibitors significantly prevent or reduce LVH due to aortic banding when antihypertensive doses were used (Clozel & Hefti, 1988; Kromer & Riegger, 1988). In spontaneously hypertensive rats (SHR) eleven months treatment with enalapril resulted in attenuation of blood pressure, limitation of cardiac hypertrophy and myocardial fibrosis (Pahor *et al.*, 1991).

Our observation that treatment with ramipril effectively prevents myocardial fibrosis and cardiac hypertrophy even in the absence of a fall in blood pressure implies tissue specific autocrine/paracrine mechanisms influenced by this ACE inhibitor. Such an antihypertrophic effect of ramipril without blood pressure reduction was also seen in remodelling of vascular structure in SHR (Friberg *et al.*, 1991). Furthermore depressed endothelium-mediated dilatation in renal hypertension recovered with treatment with the low dose of ACE inhibitor despite maintained hypertension (Goetz *et al.*, 1991).

Recently we demonstrated that a non-antihypertensive dose of ramipril in rats subjected to banding of the abdominal aorta caused regression of the cardiac mass, strongly supporting the hypothesis that Ang II itself is a tropic growth-promoting factor, independent of its haemodynamic effects (Linz *et al.*, 1989). Therefore haemodynamic changes alone could not account for the effect on cardiac hypertrophy.

In the rat heart overloaded in terms of pressure by constriction of the abdominal aorta, the adaptive increase in mass is characterized as concentric hypertrophy in which wall thickness increases without chamber enlargement (Rossi & Carillo, 1991). The stimulus for the appearance seems to be dependent on Ang II (Giacomelli *et al.*, 1976). Furthermore it was shown that Ang II stimulates collagen synthesis in vascular smooth muscle cells (Kato *et al.*, 1991).

On the other hand ACE inhibitors caused regression of cardiac hypertrophy and reduced myocardial tissue Ang II in SHR (Nagano *et al.*, 1991). This corresponds with our functional findings on isolated aortic strips of treated animals where the conversion of Ang I to Ang II was inhibited by either treatment regimen, indicating tissue ACE inhibition in the target organs. In contrast, plasma ACE activity was only inhibited in the high dose group in our one year study. Aortic strips from animals treated with high as well as low dose ramipril showed a normal relaxation after BK comparable to the relaxation of strips from sham-operated animals, whereas the endothelium-dependent relaxation in the aorta from stenosis vehicle treated animals was impaired. Reduced responses to Ang I and enhanced BK action were also observed in a one year study in rats with congestive heart failure treated with enalapril in a blood pressure lowering dose (Sweet *et al.*, 1987).

The effect of ramipril without blood pressure reduction could be explained by the interaction with tissue specific autocrine/paracrine mechanisms activated by local ACE inhibition.

Since inhibition of ACE, besides reducing Ang II formation, also increases BK concentrations, it is conceivable that enhanced endotheluim-derived BK with subsequent generation of nitric oxide (NO) (Wiemer *et al.*, 1991) contributes to the prevention of the hypertrophic response by ACE inhibitors. In the same model the Ang II receptor antagonist, Losartan (DuP 753), given in a blood pressure lowering dose was less effective on cardiac hypertrophy than ramipril in a

dose without effect on blood pressure (Linz et al., 1991). Further underlining the importance of BK is the recent observation that this antihypertrophic effect of ramipril was abolished by the BK_2 kinin receptor antagonist HOE 140 (Linz & Schölkens, 1992). A comparable sequence of events has been demonstrated in a model of neointimal proliferation in response to endothelial injury in the rat carotid artery, where the marked antiproliferative effect of ramipril was significantly reduced by the coadministration of the BK_2 kinin antagonist HOE 140 (Farhy et al., 1992). The possible participation of BK in the myocardial antihypertrophic as well as antifibrotic action of ACE inhibitors is also supported by the observation that the phosphocreatine to ATP ratio was low in hypertrophied hearts whereas hearts from treated animals showed an improved normal ratio as seen in sham-operated animals. This reduction of phosphocreatine to ATP ratio is characteristic for myocardial hypertrophy as shown for failing hypertrophied human myocardium using ^{31}P magnetic resonance spectroscopy (Conway et al., 1991). The normalization of phosphocreatine to ATP ratio by ACE inhibitor treatment might be explained by favourable metabolic effects of BK optimizing nutritional flow across the capillary wall which in turn leads to an elevated glucose uptake in the heart (Rösen et al., 1983), improving the energy situation in the hypertrophied myocardium.

As well as Ang II, the sympathetic nervous system needs also to be considered. Noradrenaline infused subcutaneously by use of miniosmotic pumps produced a concentric myocardial hypertrophy in concious rats (Newling et al., 1989). Stimulatory effects of Ang II on the facilitation of the peripheral sympathetic neurotransmission are known (Clough et al., 1981). Sen & Bumpus (1979) have shown that α-methyldopa and reserpine can each inhibit enhanced myocardial collagen synthesis in rats with genetic hypertension. The normalized plasma catecholamine content in our ramipril-treated rats supports the hypothesis that besides Ang II, catecholamines are also involved in the genesis of LVH and myocardial fibrosis.

Early observations with ACE inhibitors have shown that it is not necessary to suppress plasma ACE activity continously to keep the blood pressure of hypertensive animals as well as patients, normalized throughout the day during long-term treatment (Unger et al., 1985; Waeber et al., 1989). These observations have been taken as evidence for an antihypertensive action of ACE inhibitors not directly mediated by the blockade of the circulating RAS. Our withdrawal study confirms these observations concerning blood pressure changes in the ealier high dose RA 1 mg group and shows for the first time this phenomenon in LVH. ACE activity was normal in all groups; however, the antihypertrophic effect on LVH was still present probably via a signal set by long term ACE inhibition.

Conclusion

Long term ACE inhibition with ramipril effectively prevents cardiac hypertrophy and myocardial fibrosis even in the absence of a fall in blood pressure. This protective effect is still present after 6 months withdrawal of treatment. Interactions with autocrine-paracrine mechanisms involving decreased Ang II formation and increased BK generation with an attenuation of sympathetic activities should be considered as contributors to these beneficial cardiac effects of ACE inhibitors.

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(Received March 18, 1992
Revised July 3, 1992
Accepted July 29, 1992)